CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

Anti-human Immunoglobulin G (hIgG) U*Light*

Product number:	TRF500D	**Lot Number:**	3319540

Product Format: TRF500D: 0.2 nmoles (400µL)

TRF500M 2 nmoles (4 x1mL)

TRF500R: 20 nmoles (4 x 10mL)

Manufacturing date:	July 23, 2024	**Document version:**	1

Product Information

Application: U*Light* has been conjugated to anti-human IgG antibody. This antibody recognizes human immunoglobulins without considerations of isoforms or chain type. This toolbox can be used to either detect human immunoglobulins in samples, or may be used as a secondary antibody to "humanized" antibodies (such as FC chimeras) in TR-FRET assays.

Storage: Store product in the dark at 4 ºC.

Stability: This kit is stable for at least 12 months from the date of manufacture when stored in its original packaging and the recommended storage conditions.

Quality Control

The QC release specifications are based on spectrophotometric analysis of the labeled antibody. We certify that results meet our quality release criteria.

Labeling Ratio: 4.2
Concentration: 80.0 µg/mL (0.5 µM)

Description of the LANCE *Ultra* Assay

LANCE® and LANCE® (Lanthanide chelate excite) *Ultra* are our TR-FRET (time-resolved fluorescence resonance energy transfer), homogeneous (no wash) technologies. One antibody of interest is labeled with a donor fluorophore (a LANCE Europium chelate) and the second molecule is labeled with an acceptor fluorophore [U*Light*™ dye]. Upon excitation at 320 or 340 nm, energy can be transferred from the donor Europium chelate to the acceptor fluorophore if sufficiently close for FRET (~10 nm). This results in the emission of light at 665 nm.

Recommended Assay Conditions

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the signal.

Specific additional required reagents and materials:

The following materials are recommended:

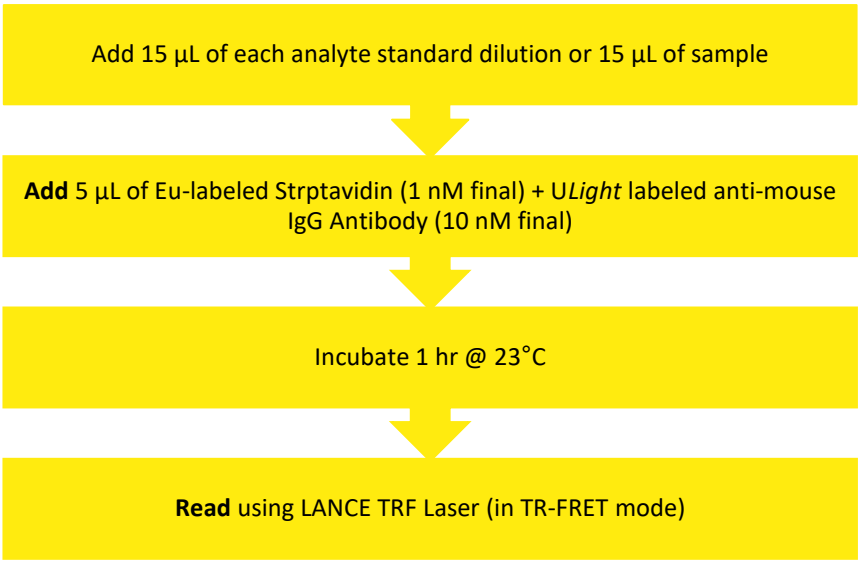
Item	Suggested source
OpitPlate-96 or OptiPlate-384	Revvity Inc.
TopSeal™-A Plus Adhesive Sealing Film	Revvity Inc.
Multilabel Plate Reader equipped with TR-FRET option, such as the EnVision®	Revvity Inc.

Example: Anti-human IgG U*Light* LANCE *Ultra* Assay

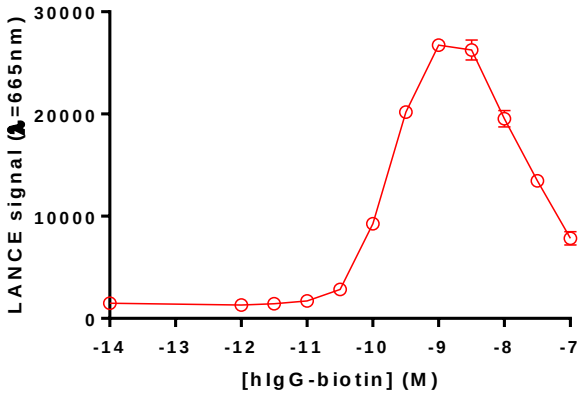
Reagents:

1. Prepare 1x Buffer: Add 2 mL of 5X PBS + 0.1% Tween 20 to 8 mL H_2O.
2. Prepare Biotin-human IgG probe standard dilutions: Dilute Biotin-human IgG Probe to 1µM with 1X PBS + 0.1% Tween 20
3. Dilute Eu-W1024 Streptavidin (1 mg) to 500 nM with 1X TSA buffer (50 mM Tris-HCl – 150 mM NaCl – 0.05% sodium azide) pH 7.4

Protocol:



Typical Product Data



Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec).
- Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute Buffer.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. LANCE *Ultra* TR-FRET assays cannot be read with the TopSeal-A Film attached. Please remove before reading.
- LANCE signal is detected using a Multilabel Reader equipped with the TR-FRET. Use an excitation wavelength of 320 or 340 nm to excite the LANCE Europium chelate. We recommend you read this assay in dual emission mode, detecting both the emission from the Europium donor fluorophore at 615 nm, and the acceptor fluorophore (at 665 nm for U*Light* dye). The raw FRET signal at 665 nm can be used to process your data.

Please visit our website for additional information on LANCE *Ultra* technology at www.revvity.com

TRF500-R Rev01

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For a complete listing of our global offices, visit www.revvity.com